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Small-scale Heterogeneity in Soil Quality Influences Photosynthetic Efficiency and Habitat Selection in a Clonal Plant

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• *Background and Aims* In clonal plants, internode connections allow translocation of photosynthates, water, nutrients and other substances among ramets. Clonal plants form large systems that are likely to experience small-scale spatial heterogeneity. Physiological and morphological responses of *Fragaria vesca* to small-scale heterogeneity in soil quality were investigated, together with how such heterogeneity influences the placement of ramets. As a result of their own activities plants may modify the suitability of their habitats over time. However, most experiments on habitat selection by clonal plants have not generally considered time as an important variable. In the present study, how the foraging behaviour of clonal plants may change over time was also investigated.
• *Methods* In a complex of environments with different heterogeneity, plant performance was determined in terms of biomass, ramet production and photosynthetic activity. To identify habitat selection, the number of ramets produced and patch where they rooted were monitored.
• *Key Results* Parent ramets in heterogeneous environments showed significantly higher maximum and effective quantum yields of photosystem II than parents in homogeneous environments. Parents in heterogeneous environments also showed significantly higher investment in photosynthetic biomass and stolon/total biomass, produced longer stolons, and had higher mean leaf size than parents in homogeneous environments. Total biomass and number of offspring ramets were similar in both environments. However, plants in homogeneous environments showed random allocation of offspring ramets to surrounding patches, whereas plants in heterogeneous environments showed preferential allocation of offspring to higher-quality patches.
• *Conclusions* The results suggest that *F. vesca* employs physiological and morphological strategies to enable efficient resource foraging in heterogeneous environments and demonstrate the benefits of physiological integration in terms of photosynthetic efficiency. The findings indicate that short-term responses cannot be directly extrapolated to the longer term principally because preferential colonization of high-quality patches means that these patches eventually show reduced quality. This highlights the importance of considering the time factor in experiments examining responses of clonal plants to heterogeneity.

Key words: Chlorophyll fluorescence, foraging behaviour, *Fragaria vesca*, habitat selection, physiological integration, source–sink hypothesis.

INTRODUCTION

Clonal plants dominate many natural communities (Kliměs *et al*., 1997). Clonal growth allows plants to form large systems consisting of a variable number of ramets located at some distance from each other, and remaining connected by stolon or rhizome internodes for a variable period of time. This kind of reproduction implies that clonal plants are likely to experience small-scale spatial heterogeneity. In many ecosystems, such small-scale heterogeneity can often be observed over distances at scales of <50 cm (Salzman, 1985; Lechowick and Bell, 1991; Caldwell and Pearcy, 1994), and is relevant for many ecological plant processes (Hartgering and Bazzaz, 1984; Price and Marshall, 1999; Day *et al.*, 2003).

A large number of studies (e.g. Hartnett and Bazzaz, 1983; Slade and Hutchings, 1987; Stuefer, 1997; Alpert, 1999; Saitoh *et al.*, 2002) have shown that connections among ramets of clonal plants by stolon or rhizome internodes allow transport of photosynthates, water and nutrients from established ramets (parent ramets) to developing ramets (offspring ramets). This physiological integration generally confers a net benefit to the newly established offspring ramets, especially under unfavourable conditions, because they can import essential resources from established ramets growing under more favourable conditions. In this way, physiological integration may buffer the clonal plants against local effects resulting from spatial and temporal changes in the quality of a habitat (Hartnett and Bazzaz, 1983; Hutchings and Bradbury, 1986; Wijesinghe and Handel, 1994). Physiological integration may also enable clonal plants to rapidly colonize and exploit higher stress patches that would otherwise be unexploitable by independent ramets or plants (Hartnett and Bazzaz, 1983; Silander, 1985; Saitoh *et al*., 2002).

It has been demonstrated that plants have a wide variety of specific receptors enabling environmental information, such as light fluxes, water pulses and the presence of nutrients, to be acquired. These receptors may influence plant growth through signal-transduction pathways (Caldwell and Pearcy, 1994). Several authors (Schellner *et al.*, 1982; Bülow-Olsen *et al.*, 1984; Eriksson, 1986; Kleijn and van Groenendael, 1999; Wijesinghe and Hutchings, 1999) have pointed out that these mechanisms for acquiring information about the environment might allow clonal plants to perceive small-scale patterns in habitat quality, and to establish new ramets in the best patches.

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In many studies on habitat selection by clonal plants, it has been stated that species may develop morphological and physiological plasticity as a strategy for foraging more efficiently for the resources they need (Wijesinghe and Hutchings, 1996, 1999; Welham *et al.*, 2002; Day *et al.*, 2003). However, most of these studies have only addressed morphological responses to resource heterogeneity, in spite of the importance of physiological processes such as photosynthesis for overall plant performance and thus for understanding the ecology of clonal plants, such as the ways in which they colonize space and overcome establishment risks.

Likewise, most previous experiments investigating the responses of clonal plants to environmental heterogeneity have used simple heterogeneity models with only two types of patch (Salzman and Parker, 1985; Slade and Hutchings, 1987; Stuefer *et al.*, 1996; Price and Marshall, 1999). In natural environments, clonal plants often have to cope with more complex habitats, where homogeneous and heterogeneous environments may differ both in the total amount of resources and in the spatial segregation of these. However, little research has been done under these more realistic scenarios of heterogeneity.

In addition, previous experiments dealing with the physiological or morphological strategies of clonal plants for resource foraging have not generally considered time as an important variable. Most experiments have investigated short-term responses, but these cannot necessarily be extrapolated to the longer term. Notably, as a result of their own activities plants may modify the suitability of their habitats. If plants selectively locate their shoots in nutrient-rich, high-quality patches, these may be deprived of nutrients and thus converted into low-quality patches. Therefore, small-scale patterns of habitat quality can be expected to change over time, with corresponding implications for plant responses. The duration of the experiment may thus affect conclusions about the relative benefits of different habitat characteristics (Fransen and de Kroon, 2001; Fransen *et al.*, 2001).

In the present study, an 11-month experiment was performed to investigate whether small-scale spatial heterogeneity of the habitat affects the pattern of placement of new ramets by the clonal species *Fragaria vesca*. The physiological and morphological responses exhibited by plants to cope with habitats of different complexity, and the consequences for plant performance in terms of biomass, new ramet production and photosynthetic activity, as estimated by chlorophyll fluorescence parameters were investigated. As far as is known, only two previous studies (Hartnett and Bazzaz, 1983; Roiloa and Retuerto, 2005) have examined the influence of physiological integration on the photosynthetic rates of clonal plants.

Specifically the following hypotheses were tested. Our first hypothesis stated that plants growing in homogenous high-quality habitats will root their new ramets randomly in the different patches, whereas plants growing in heterogeneous habitats will preferentially root their new ramets in high-quality patches. Significant differences in number of ramets among patches in the heterogeneous habitat and a random distribution of ramets among the patches of the homogeneous habitat are predicted. Our second hypothesis was that in heterogeneous habitats, low-quality patches will eventually be occupied by ramets, after conditions in the high-quality patches have deteriorated as a consequence of ramet growth. Thus, initial differences in number of ramets among patches in the heterogeneous habitat will disappear over time. Finally, assuming that physiological integration allows the redistribution of resources among interconnected ramets according to source–sink relationships (Pitelka and Ashmun, 1985; Salzman and Parker, 1985; Marshall, 1990), we hypothesized a greater integration of clones in heterogeneous habitats, with ramets acting as sources or sinks for assimilates, depending on the quality of the habitats on which they grew. Since it is widely considered that photosynthetic rates in a given plant location may be regulated, at least in part, by the net requirement for photosynthate export from that location (Sweet and Wareing, 1966; Neales and Incoll, 1968; Meyer and Whitlow, 1992), it is predicted that photosynthetic rates of ramets are driven, in part, by the steepness of the source–sink gradient, and thus, ramets in high quality patches of heterogeneous habitats will show enhanced photosynthetic activity with respect to ramets in homogeneous habitats. It is also predicted that as a consequence of the greater integration, heterogeneous habitats with patches of contrasting quality, could maintain similar biomass production to homogeneous high-quality habitats.

MATERIALS AND METHODS

Species and propagation

Fragaria vesca L., wild strawberry, is a perennial, winter-green herb of the Rosaceae family, with above-ground ramets connected by stolons. Vegetative spread allows *F. vesca* to form fairly large and long-lived clonal systems that are likely to experience significant environmental heterogeneity. All plant material used in these experiments came from 20 plants collected at a field site located 10 km south of Santiago de Compostela (north-west Spain) and propagated vegetatively for 2 years in an open-end greenhouse at the University of Santiago de Compostela before the experiment began. The plants might or might not differ in genotype. To avoid possible confounding effects of genotypes, the plants were assigned randomly to the treatments.

Experimental design

In early 2002, two types of experimental environment were constructed under natural light conditions at the field station of the University of Santiago de Compostela. Both types of environment, which will be referred to as homogenous and heterogeneous, were built into the soil of the field station, using a steel frame. This frame consisted of seven hexagonal compartments each with area 260 cm^2, arranged as six compartments around a central compartment (Fig. 1). One patch type was assigned to each compartment, in all cases with substrate depth 20 cm (thus

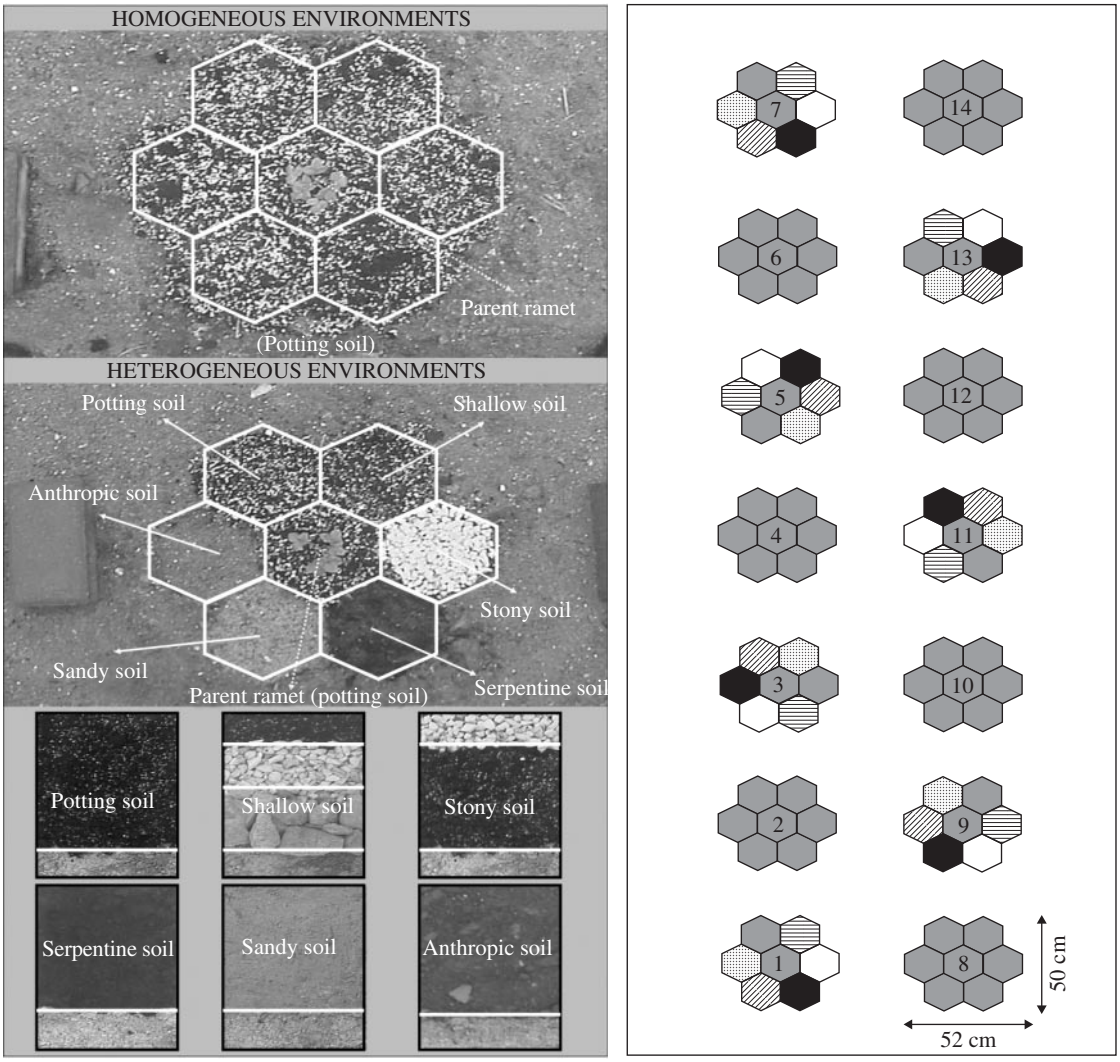

FIG. 1. Experimental layout and schematic representation of the two treatments: heterogeneity (homogenous versus heterogeneous environments) and patch type. Each homogenous and heterogeneous environment was replicated seven times, in the layout shown to the right.

substrate volume 5·2 L). The central compartment, both in homogeneous and heterogeneous environments, was filled with a 3 : 1 mixture of potting soil (Pindstrup Plus-Orange Mosebrug, Sotopalacios, Burgos, Spain) and perlite (Europerlita S.A., Barcelona, Spain). This substrate, with all main nutrients and trace elements added, may be regarded as fertile soil, and was that used previously for vegetative propagation of the plants used in the experiments, providing optimal growth conditions for *F. vesca*. All patches in the homogeneous environments were made up of this same mixture of potting soil and perlite. The six patch types assigned to the marginal compartments in the heterogeneous environments were: sandy soil (washed beach sand), stony soil (a 2-cm layer of 10–15 mm gravel above potting soil), serpentine soil (natural soil formed from parent materials with unusually high Mg and Fe content), shallow soil (a 2-cm layer of potting soil above gravel and pebbles), an anthropic soil (that of the field station) and a mixture of potting soil and perlite (like that of the central compartment). An overall assessment of the differences in total resource availability between homogeneous and heterogeneous habitats, and between the different patches of the heterogeneous habitats is given in Table 1. Although the total resource availability differed between the homogeneous and heterogeneous environments, this did not affect the contrast of our two first hypotheses. However, it was not possible to test our third hypothesis unless either the total resource amount was held constant or, as we predicted, the growth was better in the heterogeneous treatment despite having lower total resource amount.

The position of the different quality cells in the marginal compartments of the heterogeneous treatment was rotated to avoid possible confounding effects of orientation. Once the environments had been prepared the frame was removed, to allow root interactions. Each type of environment was replicated seven times. The 14 replicates were laid out in a two-row arrangement, with equal spacing between them, on a flat, fully exposed and homogeneous plot of the field station (Fig. 1). The homogeneous environmental

TABLE 1. *Mineral concentrations in homogeneous and heterogeneous environments and in the different patches of the heterogeneous environments (mg L^{-1})*

	Homogeneous	Heterogeneous						
	Total	Total	Potting	Shallow	Stony	Serpentine	Sandy	Anthropic
N	76·9	40·5	76·9	7·7	57·7	0·96	5·8	57·7
P	134·6	57·4	134·6	13·5	115·4	0·76	0·96	1·9
K	230·8	100	230·8	19·2	192·3	19·2	7·7	0·2
Mg	5·8	4279	5·8	0·38	3·8	29000	903·8	38·5
Fe	13·5	26005	13·5	1·3	11·5	136000	–	46000
Ni	–	157·5	–	–	–	1038·5	–	63·7
Cr	–	733·5	–	–	–	5000	–	134·6

nd, Not determined.

conditions of the plot allow possible confounding factors to be ruled out. As can be seen from Fig. 1, the two types of environment were interspersed regularly, as recommended by Hurlbert (1984).

In early March 2002, 14 plants with no offspring ramets were selected for size uniformity from the plant stock. Each of these plants, which will be referred to as parent ramets, was randomly assigned to one of the environment types and rooted in the centre of the central compartment, giving each patch an *a priori* equal chance of being colonized. The plants were allowed to grow for 11 months (March 2002 to January 2003). Substrates were watered daily to field capacity throughout the experiment.

Measurements

Chlorophyll fluorescence. Measurements of chlorophyll fluorescence parameters were initiated when all patches of heterogeneous and homogeneous environments had been colonized by rooted ramets. Every week during the last 3 months of the experiment, chlorophyll fluorescence parameters were measured with a portable pulse-amplitude-modulated fluorometer (MINI-PAM photosynthesis yield analyser; Walz GmbH, Effeltrich, Germany) by the saturation pulse method (see Schreiber *et al.*, 1998). Measuring light and saturating light pulses (>4000 µmol photons m^{-2} s^{-1}, 0·8 s pulse length) were applied through optical fibres at an angle of 60° and 12 mm distance from the leaf (Halogen Lamp 8V/20W, Bellaphot; Osram, Munich, Germany).

The chlorophyll fluorescence parameters recorded were as follows. The maximum quantum yield of photosystem II (PSII) was assessed by the ratio $F_v/F_m = (F_m - F_0)/F_m$ (see Bolhàr-Nordenkampf *et al.*, 1989), where F_0 and F_m are defined as minimal and maximal fluorescence yields of a dark-adapted sample, with all PSII reaction centres fully open (i.e. all primary acceptors oxidized). This parameter was measured just before dawn to ensure a dark-adaptation time sufficient for all PSII reaction centres to open. The maximum quantum yield of PSII is an estimate of the efficiency of excitation energy capture by open PSII reaction centres (Butler and Kitajima, 1975) and is correlated with the amount of carbon gained per unit of light absorbed (Bolhàr-Nordenkampf and Öquist, 1993). The effective quantum yield of PSII was calculated as $\Phi PSII = (F'_m - F_t)/F'_m$ (see Genty *et al.*, 1989), where F'_m is defined as the maximal fluorescence yield reached in a pulse of saturating light with an illuminated sample and F_t is the fluorescence yield of the leaf at a given photosynthetic photon flux density. This parameter, which measures the proportion of the light absorbed by chlorophyll that is used in photochemistry (Maxwell and Johnson, 2000), was measured on seven dates, under the ambient light conditions prevailing at midday [photosynthetic photon flux density = 1071·4 ± 23·2 µmol m^{-2} s^{-1} (mean ± s.e.), $n = 98$]. Previous studies have demonstrated that ΦPSII can be used to predict CO_2 assimilation rates precisely and quickly (Genty *et al.*, 1989; Demmig-Adams *et al.*, 1990; Edwards and Baker, 1993; Andrews *et al.*, 1995). Both fluorescence parameters were measured in three fully developed leaves per parent and offspring ramet, and the resulting mean values ($n = 3$) were used in the analyses.

Growth. At the end of the experiment, the plant material in each patch of the different environments was harvested separately, dried to constant mass at 60 °C, and weighed to determine root, leaf, stem and stolon biomass. The biomass of the stolons that they produced was included in the biomass of each ramet. The length of stolons (internode length), number of disconnected ramets, leaf areas and biomass allocation ratios (root/total biomass, aboveground/total biomass and stolon/total biomass) were also calculated. The number of disconnected ramets was calculated for all the patches at the end of the experiment. During the experiment, the production of new ramets was monitored (number of ramets and patch where they rooted) on 19 dates to identify possible preferences for colonization of particular patches.

Data analysis

The experimental design comprised two factors: 'heterogeneity' (with two levels, homogeneous and heterogeneous) and 'patch type' (six levels corresponding to six patches in the homogeneous and heterogeneous environments). Prior to analyses, all variables were examined for normality and homoscedasticity. When significant violations were found, non-parametric statistics were used. Differences between parent ramets at the end of

the experiment, in biomass, leaf area, biomass allocation ratios, ramet production and stolon lengths, were analysed by one-way analyses of variance (ANOVA) with 'heterogeneity' as factor. Differences between parent ramets in fluorescence parameters related to photosynthesis were compared by repeated-measures analyses of variance (ANOVAR), with heterogeneity as the between-subject factor. Variations among offspring ramets in biomass, leaf area, biomass allocation ratios, ramet production and stolon lengths were investigated by a profile analysis, a form of repeated measures ANOVA (Quinn and Keough, 2002), with 'heterogeneity' as the main between-subject effect and 'patch type' as the within subject effect. Due to the difficulties in achieving an adequate number of replicates (since it took a long time to colonize some patches), the differences between offspring ramets in maximum quantum yield were only evaluated for the last 5 weeks of the experiment, using profile analysis with 'heterogeneity' as the between-subject effect and 'patch type' and 'time' as within-subject factors. For the analysis of differences between offspring ramets in effective quantum yield, a sufficient number of replicates was only obtained on a single date; thus, the significance of the differences was determined by a profile analysis, such as indicated above for the analyses of offspring biomass. To test the hypothesis that 'heterogeneity' induced variation in ramet placement changes over time we used a profile analysis with 'heterogeneity' as the between-subject factor and with 'patch type' and 'time' as the within-subject factors. Differences in the number of ramets established in the different patches were analysed, separately for homogeneous and heterogeneous environments, by Kruskal–Wallis tests, with one for each of the dates on which ramet establishment was monitored. Finally, Kruskal–Wallis tests were also performed to determine whether the number of disconnected ramets differed as a function of 'heterogeneity' or 'patch type'.

The level of significance accepted was set at $P < 0{\cdot}05$. All statistical tests were performed with SPSS 11.0.

RESULTS

Photosynthetic efficiencies

It was found that the maximum and effective quantum yields of PSII in parent ramets growing under heterogeneous environments were significantly higher than in parent ramets growing under homogeneous environments (ANOVAR: $F_{1,12} = 5{\cdot}29$, $P = 0{\cdot}040$ for F_v/F_m, and $F_{1,12} = 7{\cdot}49$, $P = 0{\cdot}018$ for ΦPSII; Fig. 2). Considering the photosynthetic performances of offspring ramets, no significant effect of heterogeneity or patch type was detected (profile analysis: P always >0·099 for F_v/F_m, and P always >0·091 for ΦPSII).

Growth

Parent ramets growing in heterogeneous environments showed significantly higher investment in photosynthetic biomass (above-ground/total biomass) (ANOVA: $F_{1,12} = 5{\cdot}61$; $P = 0{\cdot}036$), mean leaf area (ANOVA: $F_{1,12} = 10{\cdot}52$; $P = 0{\cdot}007$) and stolon/total biomass (ANOVA: $F_{1,12} = 12{\cdot}70$; $P = 0{\cdot}004$) than parent ramets growing in homogeneous environments (Table 2). We found similar significant effects for stolon length (ANOVA: $F_{1,12} = 4{\cdot}99$; $P = 0{\cdot}045$; Table 2). No significant effects of heterogeneity on the rest of variables examined were detected.

Offspring ramets growing in heterogeneous environments showed a significantly higher investment in stolon biomass (profile analysis: between-subject effect $F_{1,12} = 13{\cdot}161$, $P = 0{\cdot}003$, for stolon/total biomass; Fig. 3) than those growing in homogeneous environments. However, these differences were not significant for stolon length (profile analysis: between-subject effect $F_{1,12} = 2{\cdot}373$, $P = 0{\cdot}149$). The total biomass of offspring ramets produced in homogeneous and heterogeneous environments did not differ significantly (profile analysis: between-subject

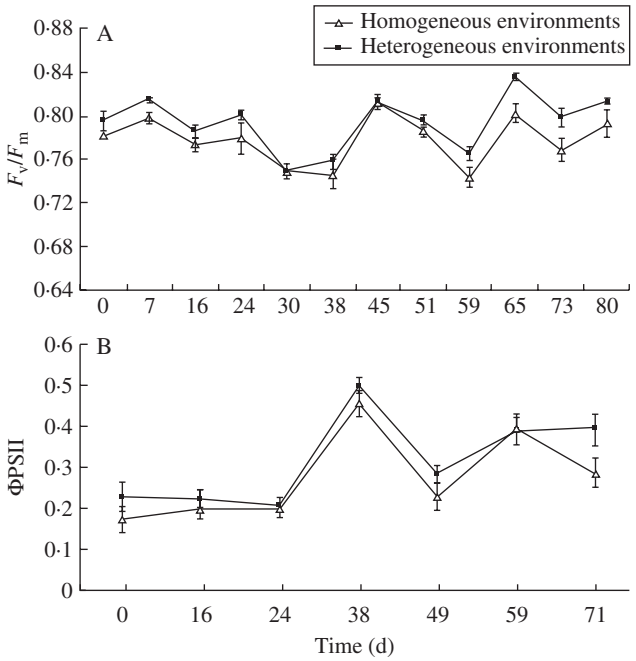

FIG. 2. Mean values (± s.e.) of (A) photosynthetic efficiency in parent ramets growing in heterogeneous and homogeneous environments, as estimated by the maximum quantum yield of PSII (F_v/F_m) (ANOVAR indicated significant between-subjects effects: $F_{1,12} = 5{\cdot}29$, $P < 0{\cdot}05$), and (B) the effective quantum yield of PSII (ΦPSII). ANOVAR indicated significant between-subjects effects: $F_{1,12} = 7{\cdot}50$, $P < 0{\cdot}05$.

TABLE 2. *Mean (± s.e.) and ANOVA* P*-values for above-ground : total biomass ratio, mean leaf area (mm²), stolon : total biomass ratio and stolon length (cm) of parent ramets growing in homogeneous or heterogeneous environments*

	Homogeneous	Heterogeneous	*P*-values
Above-ground : total biomass	0·582 ± 0·051	0·746 ± 0·057	0·036
Mean leaf area (mm^2)	1089·28 ± 176·27	2014·02 ± 265·35	0·007
Stolon : total biomass	0·031 ± 0·007	0·113 ± 0·015	0·004
Stolon length (cm)	12·67 ± 4·16	22·41 ± 1·30	0·045

In all cases ANOVA indicated significant differences between homogeneous and heterogeneous environments (d.f. 1,12).

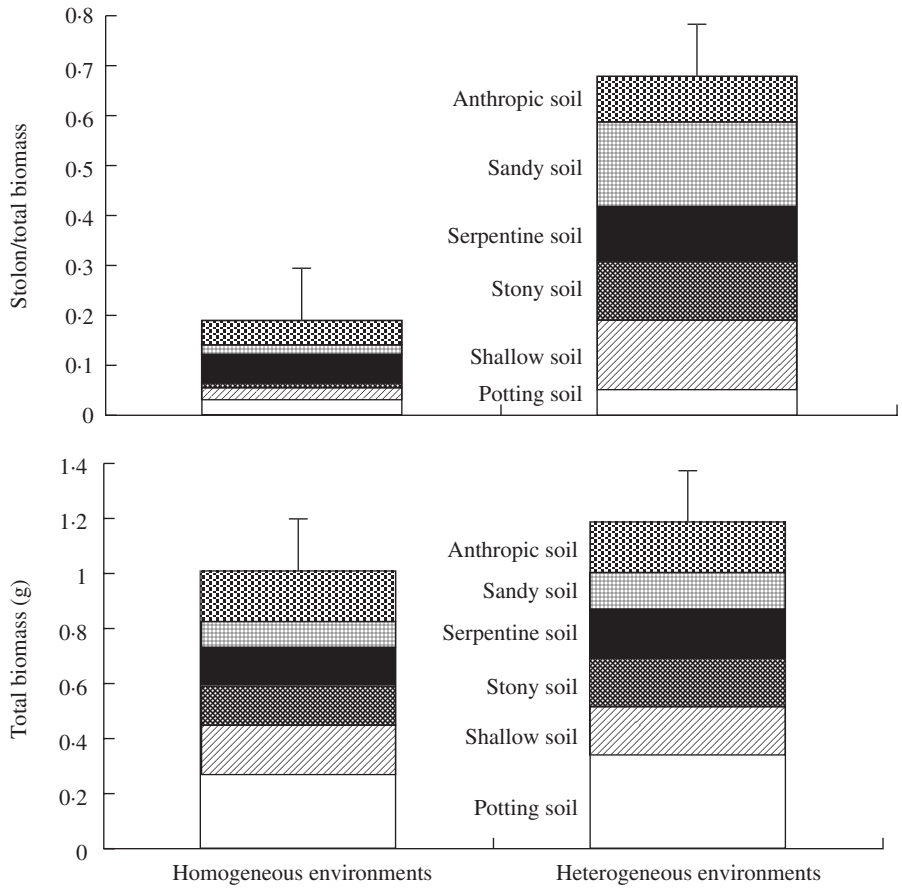

FIG. 3. Mean values (± s.e.) for stolon : total biomass ratio and total biomass (g) of offspring ramets growing in homogeneous and heterogeneous environments. Profile analysis indicated significant differences between the two types of environment for stolon : total biomass ratio (between subject effect: $F_{1,12} = 13{\cdot}161$, $P = 0{\cdot}003$), but not for total biomass (between subject effect: $F_{1,12} = 0{\cdot}223$, $P = 0{\cdot}646$).

effect $F_{1,12} = 0{\cdot}223$, $P = 0{\cdot}646$; Fig. 3). No significant effects were detected on the rest of the growth variables studied.

No significant effect of heterogeneity or patch type was found on the number of disconnected ramets (*P*-values for Kruskal–Wallis tests always >0·094).

At the end of the experiment, there were no offspring ramets in the central patch of any of the environments. Some stolons were long enough to leave the experimental areas. These stolons were left to grow outside but they were not included in the analyses. In any case, we did not detect differences between homogeneous and heterogeneous environments in the biomass outside the area ($F_{1,82} = 0{\cdot}075$, $P = 0{\cdot}785$).

Habitat selection

The significant interaction term between 'heterogeneity', 'patch type' and 'time' indicates that ramet placement in the patches differed between homogeneous and heterogeneous environments over time (profile analysis: $F_{80,960} = 1{\cdot}556$, $P = 0{\cdot}002$). The results show that in heterogeneous environments the parent ramets did not establish new ramets in any of the patches until April 2002 (Fig. 4). Between April and May, parent ramets only established new ramets in the highest-quality patches, the potting soil patches. Between June and December ramets additionally colonized other types of patches. Kruskal–Wallis tests for each of the 11 data sets obtained in that period detected significant effects of patch type on the number of ramets established in the heterogeneous environments, with more ramets in the potting soil patches (Fig. 4). Parent ramets did not establish offspring ramets in all six surrounding patches until the end of August. Kruskal–Wallis tests for the two data sets obtained in January did not detect any significant effects of patch type on the mean number of ramets established in the different patches of the heterogeneous environments (see Fig. 4).

The process of colonization of new patches was faster in homogeneous than in heterogeneous environments (Fig. 4). At the start of July, parent ramets had already established at least one ramet in each of the patches that made up the homogeneous environments, about 2 months earlier than in the heterogeneous environments. Although Fig. 4 seems to suggest a possible effect of orientation on the number of ramets established in the patches of the homogeneous environments (four contiguous patches showed more ramets), Kruskal–Wallis tests for the relevant data sets (i.e. homogenous environments, on dates on which ramets had been established) did not detect any significant effect

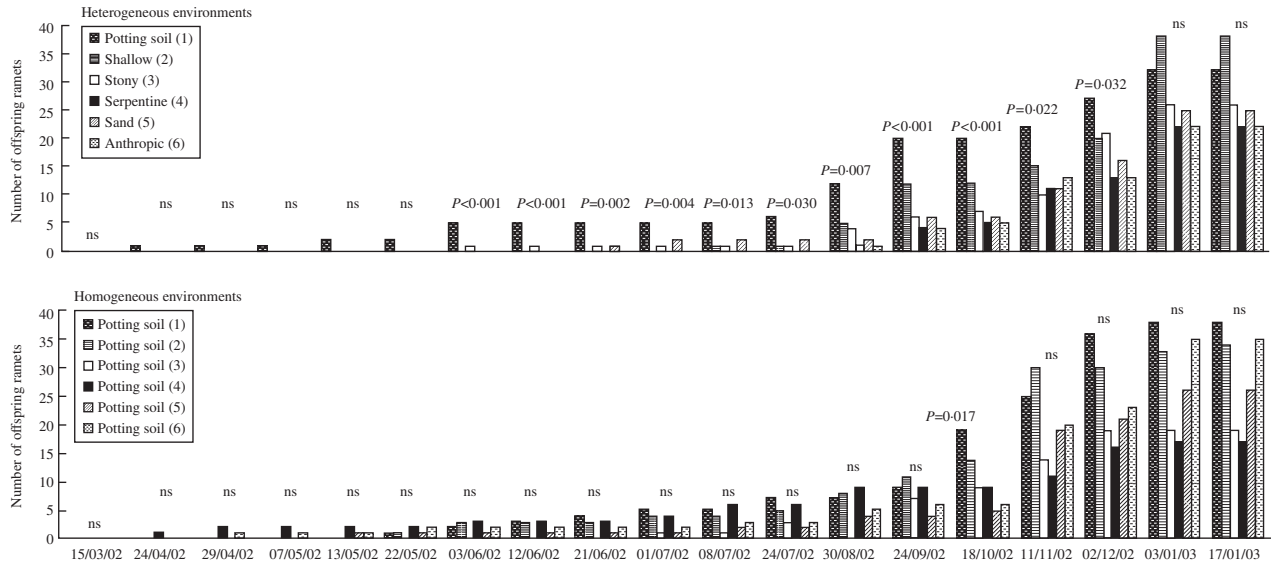

FIG. 4. Time-course of the colonization of patches in homogeneous and heterogeneous environments by ramets. For each date, columns show the total number of offspring ramets (n = 7 replicates) established in the different patches making up the heterogeneous and homogeneous environments. P-values for Kruskal–Wallis tests are shown (ns = non-significant differences). Profile analysis: $F_{80,960} = 1{\cdot}556$, $P = 0{\cdot}002$, for the heterogeneity × patch type × time interaction effect.

of patch position on the number of ramets established, with the single exception of October. To confirm the results of the Kruskal–Wallis test, circular statistics were used to test the uniformity of distribution of ramets around a circle in the homogeneous environments. The Rayleigh test for grouped data (grouping interval 60°) (Batschelet, 1981; Zar, 1984) applied to the mean number of ramets in the different patch positions (six positions, $n = 7$ replicates) confirmed that the ramets were uniformly distributed around the circle in the homogeneous environments (P always >0·2).

The total number of ramets established on the different dates on which new ramets were present did not differ significantly between homogeneous and heterogeneous environments, except on July 24 (Kruskal–Wallis test: $\chi^2 = 4{\cdot}442$, d.f. 1, $P = 0{\cdot}035$).

DISCUSSION

Regarding our first hypothesis of a preferential location of ramets in high-quality habitats, the results of this study confirm our predictions. It was found that in heterogeneous environments, *F. vesca* first rooted new ramets in the highest-quality patches (Fig. 4), whereas in homogeneous environments ramets were rooted randomly on the different patches (Fig. 4). These results did not reveal the mechanism for ramet location, whether active foraging or delay (or even suppression) of root formation. Some authors have suggested a mechanistic explanation for the selective placement of ramets. Wijesinghe and Hutchings (1999) observed that plants of the stoloniferous *Glechoma hederacea* initially extend some ramets into both favourable and unfavourable patches, apparently in order to gauge local habitat quality. In line with this, Schellner *et al.* (1982) and Eriksson (1986) have suggested that stolon tips of clonal plants might sense areas with high nutrient concentrations, water availability or light flux, increasing the probability of placing ramets in favourable sites. Moreover, Salzman (1985) and Oborny (1994) have interpreted that the preferential colonization of certain habitats is the result of a facultative reduction by plants of resource allocation for growth in unfavourable habitats. Soil type-dependent differences in air humidity may be a plausible mechanism to explain the variation in initial ramet placement. Organic potting compost inherently holds more water, thereby increasing the humidity experienced by plants close to the soil surface. High humidity, in turn, may stimulate root formation.

Our second hypothesis was that in the heterogeneous environments, initial among-patch differences in the number of ramets will disappear over time. Our results confirm this prediction. From June to December significantly higher numbers of rooted ramets were consistently observed in the potting soil patches of the heterogeneous environments. This pattern was no longer observed at the end of the experiment, in January, when no significant effects of patch type on number of ramets rooted in the heterogeneous environments were detected. The accumulation of biomass in the different patches can be expected to have followed a similar trend. At the end of the experiment, no significant effect of patch type on the total biomass of offspring ramets was observed. A plausible explanation is that as a result of the preferential allocation of ramets to the high-quality patches in the heterogeneous environments, these patches become low-quality patches by overcrowding. At their maximum density the ramets cast shade or, having achieved optimal ramet spacing, allocate to spreading new ramets elsewhere. Mechanisms that tend to minimize competition among parts of the same

clone have been described. Gruntman and Novoplansky (2004) have suggested that the clonal system may recognize itself and avoid competition by starting growth in other patches. Although previous studies have shown that the potential benefits of selective root placement in heterogeneous soils may be reduced by patch depletion (van Vuuren *et al.*, 1996; Frasen *et al.*, 1998; Hodge *et al.*, 1999), nutrient depletion is discarded as an explanation because at the end of the experiment the roots did not occupy the whole soil volume. In fact, a considerable volume of soil (5200 mL per patch) was used. This volume of soil is clearly enough for maintaining *F. vesca* growth during the 11 months of the experiment. Whatever the explanation, the results of the present study support the model of habitat selection proposed by Fretwell (1972) for animal populations. This simple model assumes that individuals can move into habitats without restrictions ('ideal free distribution') and that the suitability of habitats is not constant but affected by the density of the individuals, so that overcrowding reduces suitability. The model predicts that when density is high in good habitats, good and poor habitats will have equal suitability. Given that plants can change the quality of patches, with corresponding implications for plant responses, the results of the present study, as well as the results of others (Fransen and de Kroon, 2001; Fransen *et al.*, 2001), point to the importance of considering time as a key factor when investigating the foraging behaviour of clonal plants.

Our third hypothesis was that parent ramets will send resources to offspring ramets in poor-quality environments and that, in line with this, photosynthetic efficiency will be higher in parent ramets growing in heterogeneous environments than in parent ramets growing in homogeneous environments. The results of the present study show that the maximum and effective quantum yields of PSII in parent ramets growing in heterogeneous environments were significantly higher than in parent ramets growing in homogeneous environments. It can be argued that these differences, although statistically significant, are quantitatively quite small. However, they may accumulate throughout development to contribute substantially to differences in carbon gain and to produce large differences in fitness, as Thomson *et al.* (2003) have suggested. These results, along with the maintenance of the photochemical activity of offspring ramets growing in the low quality patches of the heterogeneous environments, support our hypothesis of greater integration of clones in heterogeneous environments, and are in line with results of Alpert *et al.* (2003), showing that a higher potential for resource sharing is selected for in more heterogeneous habitats. It is suggested that the observed enhancement in photosynthetic efficiency allowed parent ramets in heterogeneous environments to attend to the increased photosynthate demand from 'dependent' offspring, especially those growing in low-quality patches. The present data are also consistent with the findings of Hartnett and Bazzaz (1983), who reported an enhancement of photosynthetic rates in illuminated ramets connected to shaded ramets of *Solidago canadensis*, and with the findings of Roiloa and Retuerto (2005), indicating an enhancement of photosynthetic activity in parent ramets of *F. vesca* supporting offspring ramets growing under water-stress conditions. Day *et al.* (2003) have suggested that physiological responses to heterogeneity, such as increased rates of uptake of nutrients, may explain why some species produce more biomass in heterogeneous environments than in homogenous environments, as reported by some previous authors (Birch and Hutchings, 1994; Einsmann *et al.*, 1999). Other kinds of physiological response to heterogeneity, such as the increased photosynthetic efficiencies reported in our study, may be also important for explaining the surprising result that plants in heterogeneous but mostly lower-quality habitats produce as much biomass as plants in uniformly high-quality homogeneous environments.

Changes in CO_2 assimilation measured by gas exchange correlate significantly with fluorescence-based indices of the effective quantum yield of PSII photochemistry (Genty *et al.*, 1989; Demmig-Adams *et al.*, 1990; Edwards and Baker, 1993; Andrews *et al*., 1995). Gas exchange and fluorescence-based indices measure different components of the photosynthetic process associated with CO_2 fixation (carbon reactions) and with light-harvesting and electron transport (light reactions), respectively. The electrons transferred along the photosystems provide energy and substrates for carboxylation, but a certain proportion may also be consumed in processes that do not result in carbon fixation, mainly photorespiration (Krall and Edwards, 1992; Björkman and Demmig-Adams, 1995). The present study provides evidence of how the light reactions of the photosynthetic process are affected by physiological integration, and strongly suggest that CO_2 fixation may also be affected.

In addition to greater photosynthetic efficiencies, parent ramets growing in heterogeneous environments also exhibited morphological responses to heterogeneity. Parent ramets in heterogeneous environments showed a significantly higher investment in photosynthetic biomass (aboveground/total biomass) and stolon/total biomass, produced longer stolons, and had higher mean leaf size than parent ramets in homogeneous environments. Morphological plasticity in response to heterogeneity may reflect the placement of structures to ensure efficient foraging for resources (Hutchings and de Kroon, 1994; Alpert and Stuefer, 1997; Cain, 1994; Einsmann *et al*., 1999), and can therefore be considered an adaptive response to heterogeneity (Jain, 1979). Several authors have reported that ramets growing under unfavourable conditions generated longer internodes, but in smaller numbers, than ramets established in favourable areas; they interpreted this response as a strategy for escape from unfavourable conditions (Hartnett and Bazzaz, 1983; Sutherland and Stillman, 1988; Welham *et al*., 2002; Macek and Lepš, 2003). A higher investment was also observed in stolon biomass in offspring ramets growing under heterogeneous conditions. However, there were no differences in stolon length. This should mean thicker stolons, which could be an indication for more transport capacity. For the offspring ramets, the escape theory does not seem to work.

In terms of total biomass and production of new ramets, the putatively different foraging strategies to exploit

heterogeneous environments (preferential allocation of ramets to high-quality patches) and homogeneous environments (random allocation of ramets to patches) produced similar results.

In conclusion, the present study confirms that *F. vesca* shows physiological and morphological responses enabling more efficient foraging for resources in heterogeneous environments. Perhaps the most interesting result of the study is that it demonstrates the benefits of physiological integration in terms of feedback regulation of photosynthesis. This is an important aspect of physiological integration that deserves to receive more attention, since it may be important for explaining the overall better performance of clonal plants in heterogeneous environments. This study shows that the greater integration in heterogeneous environments allows maintaining similar biomass in the patchy treatment (despite having lower total resource amount) and in the homogeneous environment.

The present study also highlights the importance of time in experiments examining responses of clonal plants to heterogeneity. Most previous experiments of this type have tended to be short, and have not considered that clonal plants by their own expansion may themselves reduce heterogeneity among microsites. The present study indicates that short-term responses cannot be directly extrapolated to the long term.

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